Section I: Provenance & Release

1. Who did the actual work to acquire these measurements?

   Orhan Sayın, Muhammed Fatih Aktaş, Osman Faruk Tereci, Mustafa YILMAZ, Muhammed Fatih Aktaş, Turan KAYA, Abdullah Uçar, Furkan Sacit Ceylan, Mikail Doğan, Safa Tapan, Mustafa Elitok, Furkan Beştepe, Fethi Önal

2. What other people should be credited for these measurements? (i.e., who would be an author on any resulting publication. For example, your faculty advisor may have helped design the protocols that you ran.)

   Mehmet Gündüz, Esra Gündüz, Ömer Faruk Hatipoğlu, Muradıye Acar, Sadık Çiğdem

3. On what dates were the protocols run and the measurements taken? (this will often be a range of dates; make sure you say which data was taken at what times.)

   Protocols were run between 15 August and 27 August.

   All results got at 27 August.

4. Do all persons involved consent to the inclusion of this data in publications derived from the iGEM interlab study?

   All of us.

Section II: Protocol

1. What protocol did you use to prepare samples for measurement?

   Digestion:

   500 ng DNA for each sample

   (safa abiye soruldu) Fast Digest Buffer(Thermo Scientific)

   (safa abiye soruldu) Fast Digest Restriction enzymes

   40 minutes 37°C

   20 minutes 80°C

   Ligation:

3 ul for each sample

1 ul backbone

2 ul T4 DNA ligase Buffer(Thermo Scientific)

1 ul T4 DNA Ligase (Thermo Scientific)

60 minutes 22°C

5 minutes 70 °C

Transformation:

Take bactery from -80°C into ice

Wait 10 minutes

Take 2 ul DNA and 50 ul bactery(in 1.5 eppendorf)

Wait 5 minutes in ice

Heat shock (37-42°C) 30 seconds

Put samples into ice for 2-3 minutes

Add 200 ul lb which is non antibiotic and wait 30 minutes at 37 °C

And put samples to plates

Plasmid Isolation:

Put lysis and neutralization solutions into etüv at 37 °C

Transfer liquid cultures into 2ml eppendorfs.

Centrifuge 10 mins at 14000 rpm

Put out supernatant

Add 250 ul resuspention solution

Vortex untill all pellet is diluted

Add 250 ul lysis solution and invert

Wait 2-3 mins

Add 350 ul neutralization solution and invert(see something like cotton)

Centrifuge 10 mins on 14000 rpm

Transfer supernatant into spin columns

Centrifuge 1 min on 14000 rpm

Put out supernatant

Add 500 ul wash solution

Centrifuge1 min on 14000 rpm

Put out supernatant

Add 500 ul wash solution

Centrifuge1 min on 14000 rpm

Transfer filters to 1,5 ml eppendorfs

Add 50 ul nfw exactly in the middle of filter

Wait 2 mins

Centrifuge 2 mins on 14000 rpm

Transfer them into pcr tubes

And measure

2. What sort of instrument did you use to acquire measurements?

   - What is the model and manufacturer?

     Thermo Scientific

     Varioskan Flash

   - How is it configured for your measurements? (e.g., light filters, illumination, amplification)(ölçüm yapımı?)

3. What protocol did you use to take measurements?

   We cantrifuged liquid cultures which are waited 16 hours in lb with apropriate antibiotic.

   Then we put out supernatant and added RIPA buffer that can do dilution.

   The we smashed these with sonicator.

   Then we put samples in varioskan flash device.

   And we chose flourescence measurement.

   Then we got results.

4. What exactly were the controls that you used?

   We used nuclease free water. We measured it with our interlab study samples.

5. What quantities were measured? (e.g., red fluorescence, green fluorescence, optical density)

   Red flourescence, green flourescence and optical density all of these were measured.

6. How much time did it take to acquire each set of measurements?

   About 30 minutes

7. How much does it cost to acquire a set of measurements?

We couldn't calculate how muc it costs.

8. What are the practical limits on the number or rate of measurements taken with this instrument and protocol?

We didn't use to know to use this device well. To learn took much time.

Section III: Measured Quantities

1. For each type of quantity measured (e.g., fluorescence, optical density), report on the following:

2. Units:

   - What are the units of the measurement? (e.g., meters, molecules)

     RFU: relative flourescense unit

   - What is the equivalent unit expressed as a combination of the seven SI base units? (http://en.wikipedia.org/wiki/SI_base_unit)

     RFU/ug of total

3. Precision:

   - What is the range of possible measured values for this quantity, using your instrument as configured for these measurements? (e.g., a meter stick measures in the range of 0 to 1 meter)

     http://www.biotek.com/resources/articles/green-fluorescent-proteins.html

     We learnt gfp exitation emission values from this site. With reference to we measured our samples.

   - Is the precision the same across the entire range? If not, how does it differ?

     Yes

   - How did you determine these answers?

     Our instuctors and advisors and some sites helped us to get answers.

4. Accuracy:

- When was the instrument last calibrated?

Automatic runtime calibration is always automatically performed at the beginning of the protocol execution.

- How was the instrument calibrated

The instrument also performs calibrations during protocol execution if it does not violate the timing requirements of the assay.

In fluorometry the automatic calibration expire period is 10 minutes. Recalibration is performed 7,5 minutes after the previous calibration depending on the measurement procedure. If a measurement is performed 10 minutes after the previous calibration, a warning appears about calibration validity. This may happen if an assay takes a long timr to execute and there is no sufficiant time slot to perform calibration during protocol execution. However, the warning does not mean that the measurement failed, only that the accuracy of the results may have suffered

Section IV: Measurements

1. For each sample, report:

- the identity of the sample

1st device: I20260

2nd device: BBa_J23101 + BBa_E0240 in pSB1C3 bacbone

3rd device: BBa_J23115 + BBa_E0240 in pSB1C3 bacbone

- each quantity directly measured

- each quantity derived from measurements (e.g., fluorescence/OD)

	Protein conc.
Blank	-0,010
I20260 3	55,522
I20260 2	40,079
I20260 1	40,194
J23101 1	30,885
J23101 2	32,311
J23101 3	33,331
J23115 1	41,174
J23115 2	36,254

2. For each group of replicates, report:

- the identity of samples in the set

1.For I20260 ; we have 3 replicate.

2 For BBa_J23101 + BBa_E0240 in pSB1C3 bacbone: we have 2 replicate

3. For BBa_J23115 + BBa_E0240 in pSB1C3 bacbone : we have 3 replicates

- which, if any, of the samples are excluded and why

For 3rd device we excluded 1 sample. Because that result was wrong.

But this wrong result caused from personal fault at plasmid isolation time.

- the mean and standard deviation for each quantity measured or derived

Standart deviation graphics show us our interlab study replicates are consistent each other.